12 and the descriptions of Figs. 1 and 3. If Applicant is still deemed non-compliant with any portion of 37 C.F.R. §§ 1.821 through 1.825, Applicant requests that the reasons for non-compliance be enumerated with specificity so that they may be corrected. Regarding the Abstract, that the application as filed contained an Abstract of the Disclosure as required by 37 C.F.R. § 1.72(b) as can be seen in the enclosed cover page from the PCT publication of this application and from page 19 of the application. A copy of page 19 of the application is enclosed. Nonetheless, Applicant has enclosed a copy of the Abstract of the Disclosure on a separate sheet for entry if original page 19 cannot be located.

In response to the Examiner's claim objections, Claims 16 and 17 have been amended to remove multiple multiple dependencies. Applicant notes with appreciation the Examiner's itemization of informalities in Claims 1-18 and, as a result, the Applicant has amended those claims to provide proper articles and correct spellings.

**Response to §112 Rejections**

Claims 1-18 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that was not described in the specification in such a way so as to reasonably convey to one skilled in the relevant art, that the inventor was in possession of the claimed invention at the time the application was filed. In response to the aforementioned rejection of Claims 1-18, Applicant respectfully submits that as a result of the amendments to the claims and for the reasons set forth below in detail, this rejection is now obviated.

The Applicant respectfully submits that the specification, as written, along with the material incorporated by reference, demonstrate that the inventor was in possession of the claimed invention at the time the application was filed. The Applicant submits that the specification teaches plastid transformation vectors that encode for a cytotoxic antimicrobial peptide. Antimicrobial peptides ("AMPs") are well described in Zasloff U.S. Patent Nos. 5,643,876 and 4,810,777, Haynie U.S. Patent No. 5,847,047, Olsen et al.

U.S. Patent No. 6,143,498 and Kim et al. U.S. Patent No. 6,183,992. These references were incorporated by reference in the Applicant's specification. At the Examiner's request, the Applicant can include copies of the aforementioned patents and/or directly incorporate material. However, Applicant contends this is unnecessary since AMPs are well-known. The Applicant's unique feature is not the different antimicrobial peptides. Rather the unique aspect of Applicant's invention is stably integrating a gene which encodes an antimicrobial peptide into the plastid genome of plant cells, wherein the resulting antimicrobial peptide is transcribed in the plastid and transferred into cytosol when a microbe infects the plant cell.

By describing and disclosing a particular amino acid sequence, the Applicant has specifically defined the genes and DNA sequences that are capable of coding for the desired amino acid sequence. Attention is directed to page 3, lines 10-12, which discusses the unique structure of antimicrobial peptides. The Examiner is asked to consider page 3, lines 12-17 of the Applicant's specification, which discusses the high sequence homology between the various cytotoxic antimicrobial peptides.

> It has been shown that magainin, cecropins, and bombinin oligopeptides form similar secondary structures described as an amphiphilic helix (Kaiser et al. Annu. Rev. Biophys. Biophys. Chem 16, 561-581, 1987). These peptides with *a*-helical structures are ubiquitous and found in many organisms. They are believed to participate in the defense against potential microbial pathogens. One of the first biocidal oligopeptides to be isolated from natural sources was bombinin and is described by Csordas et al. (Proc. Int. Symp. Anim. Plant Toxins, 2, 515-523, (1970)). Csordas teaches significant sequence homology between bombinin and melittin, another antimicrobial peptide, isolated from bee venom. Although pollen from plants that exhibit maternal inheritance contain metabolically active plastids, the plastid DNA is lost during pollen maturation (Helfetz, 2000). Despite the potential advantage of plastid reproduction of AMPs, it was not obvious that AMPs would be produced in this manner. Prior to the patent application there were no published reports of expression of AMPs in plant plastids.

These references show that the high degree of homology along with the unique

structural characteristics of antimicrobial peptides allows one skilled in the art to find all of the various amino acid sequences which comprise the cytotoxic antimicrobial peptides, and their resulting DNA sequences. While Applicant acknowledges that even one change in an amino acid sequence can have an impact on the resulting peptide, Applicant contends that as a result of the unique structural and phenotypical characteristics of cytotoxic antimicrobial peptides, one skilled in the art could readily ascertain whether the homologous amino acid sequence was a cytotoxic antimicrobial peptide. Furthermore, Applicant need not show that every AMP is known, or that his invention operates with every known AMP, provided it operates with those AMPs tested, and that other AMPs are sufficiently similar in structure that one skilled in the art would be led to believe those other AMPs would also work in applicant's invention. This has been done. There is no indication why any known AMPs, or any which might be found in the future would not be operative in Applicant's claimed invention.

The Action also asserts that the Applicant has not shown selectable marker sequences in the absence of an antibiotic. Applicant respectfully asserts that such a showing is unnecessary. Antibiotic-free selectable markers in plants are well-known, as evidenced by U.S. Patent No. 5,633,153 to Ursin, issued May 27, 1997. Ursin shows antibiotic-free selectable markers. These markers are aldehyde dehydrogenases, including betaine aldehyde dehydrogenase (BADH). Sequences for genes encoding the aldehyde dehydrogenases are also shown there. As the particular antibiotic-free selectable marker for applicant's invention is a matter of choice, and since such markers (and the sequences of genes encoding them) are well known, applicant believes no specific recitation is necessary.

Claims 1-18 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that was not described in the specification in such a way so as to enable one skilled in the art to which pertains, or with which it is most nearly connected, to make and/or use the invention. The Applicant submits that the

specification as written teaches plastid transformation vectors that are stably integrated into a variety of plant species, and plants transformed with such vectors, and the proper method for stably transforming a plant using the aforementioned vectors.

The prevailing standard for determining whether the specification meets the enablement requirement is whether the experimentation needed to practice the invention is undue or unreasonable. *See* Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916). As long as the specification discloses at least one manner for making and using the claimed invention that bears a reasonable correlation to the active scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied. *In re* Wands, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988).

Applicant submits that the specification, as written, fully enables one skilled in the art to practice the invention without undue experimentation. On page 9, lines 9-16, applicant clearly sets forth a vector useful for the present invention. The vector consists of the 16s-rRNA promoter, the aadA gene, the MSI-99 gene (encoding a 22 amino acid AMP), and the psbA terminator. The flanking sequencers were from the petunia plastid genome. These sequences are well-known and available to one skilled in the art. Thus, contrary to the assertion in paragraph 4 on page 5 of the action, the application shows a plastid transformation vector comprising an expression cassette comprising a plastid promoter (16S rRNA), a selectable marker (aadA), cytotoxic antimicrobial peptide (MSI-99), transcription termination sequence (psbA) and flanking sequences (from the petunia plastid). The sequences used in this vector are well-known and no specific recitation is necessary, as they are only exemplary of the invention and inasmuch as one of ordinary skill in the art would be able to find and assemble them without undue experimentation, once the invention has been disclosed.

Regarding the broad usefulness of the vectors of the present invention, across a variety of plant species, attention is directed to Daniell et al., 1998 and Guda et al (2000) describe in great detail construction of broadly applicable vectors for chloroplast

transformation. DeCosa et al 2000 and Kota et al. (1999) also describe in detail chloroplast vector constructions and characterization of transgenic plants.

Furthermore, U.S. Patent Nos. 5,693,507 and 5,932,479 teach a multitude of transformation vectors that are capable of stably transforming a variety of plant species as set forth in the specification. Furthermore, the '479 patent teaches an expression vector which comprises control sequences comprising a chloroplast promoter functional in chloroplast, a transcription termination sequence whereby there is provided expression of a coding sequence in a plant chloroplast and downstream of the promoter, a restriction site for cloning the coding sequence. This is information with which one skilled in this art would be familiar.

The Examiner is invited to consider column 4 line 63 to column 5 line 7 of U.S. Patent 5,563,507 which states:

> The expression cassette generally includes the following minimum components, the 5' untranslated region from a microorganism gene or chloroplast gene such as psbA which will provide for transcription and translation of a DNA sequence encoding a polypeptide of interest such as genes which provide for herbicide resistance or encode insecticidal proteins; and a translational and transcriptional termination region such as a 2' inverted repeat region of a chloroplast gene that could stabilize RNA of introduced genes, thereby enhancing foreign gene expression.

Column 6 lines 47 to 57 of that patent further states:

> In preparing the expression cassette, the various DNA sequences may normally be inserted or substituted into a bacterial plasmid. Any convenient plasmid may be employed, which will be characterized by having a bacterial replication system, a marker which allows for selection of the bacterium, and generally one or more unique, conveniently located restriction sites. The plasmids, or vectors, may include such vectors a pUC, pBR322, pBlueScript, and pGEM, the particular plasmid being chosen based on the nature of the markers, availability of convenient restriction sites, copy number and the like.

Example 1 of the '507 patent teaches construction of chloroplast expression vectors:

> A series of chloroplast expression vectors has been constructed using the promoter selection vector pKK232-8 (Pharmacia), which is a pBR322 derivative containing a promotorless cat gene. Restriction fragments of chloroplast DNA (cpDNA) containing the entire promoter region and 5' untranslated region of the psbA gene from spinach (pMP450, a gift from Wilhelm Gruissem, University of California, Berkeley) pHD306 or pea (pPPBX10218, a gift from John Mullet, Tex. A&M University) pHD312 or, alternatively, the rbcL and atpB promoter region from maize (pPBI1443, a gift from Antony Gatenby, E.I. DuPont de Nemours & Co., Wilmington, Del.) PHD103 have been individually inserted into the multiple cloning site (MCS) that exists 5' proximal to the promoterless cat gene.

For further information as to the knowledge of one skilled in the art of plant plastid transformation vector construction, attention is directed to Daniell (1997) Methods in Molecular Biology 62: 463-489, which describes in detail all methodologies for vector construction, bombardment and characterization of chloroplast transgenic plants. The description of methodologies in Methods is very detailed and shows that one skilled in the art would be familiar with plant plastid transformation production.

The specification provides extensive instructions for identifying in an appropriate manner a nucleic acid sequence which encodes for a desired antimicrobial peptide of interest. Heifetz (2000, Biochimie 82: 655-666) teaches that complete plastid genomes have been sequenced from a variety of plant and algal species providing a wealth of information regarding conservation of reading frames and regulatory sequences. Plastid-encoded messages have also been found to be regulated post-transcriptionally over an unusually broad range. Further, there has been a publication of the complete DNA sequences and genomic maps of at least fourteen different plant species. The following complete chloroplast genome sequences are already available in Genbank:

*Marchantia polymorpha* 121,024 kbp
*Nicotiana tabacum* 155,844 kbp
*Oryza sativa* 134,525 kbp
*Epifagus virginiana* 70,028 kbp
*Pinus thunbergii* 119,707 kbp
*Zea mays* 140,387 kbp
*Arbidopsis thaliana* 154,478 kbp
*Triticum aestivum* 134,540
*Euglena gracilis* 143,172 kbp
*Cyanophora paradoxa* 135,599 kbp
*Odontella sinensis* 119,704 kbp
*Porphyra purpurea* 191,028 kbp
*Chlorella vulgatis* 150,613 kbp
*Mesostigma viride* 118,360 kbp

As a result of this information one skilled in the art could easily find appropriate antimicrobial DNA sequences encoding for an antimicrobial peptide, which are well known in the art, and insert such sequences into a plastid of interest. Attention is also directed to DeGray et al. Expression of an Antimicrobial Peptide via the Chloroplast Genome to Control Phytopathogenic Bacteria and Fungi, *Plant Physiology*, November 2001, Vol. 127, pp 852-862 (copy enclosed), which demonstrates the expression of MSI-99 via the chloroplast genome to obtain high levels of expression in transgenic tobacco plants.

Applicant respectfully traverses the Examiner's citation to Okamoto et al. (1999, Plant Cell Physiol. 39:57-63) and Allefs et al. (1995, Am. Potato J. 72:437-445). Both the Okamoto and Allefs references taught insertion of an antimicrobial peptide into the ***nuclear*** genome of their respective plants. Applicant respectfully submits that the reason the peptides fail to retain biological activity was owing to their nature of being transcribed in the nucleus and retained within the cytosol of the plant, rather than what the Applicant's have discovered, which is transcribing and retaining the peptide within the chloroplast genome. Further, the Applicant has fully disclosed transcribing a peptide or antimicrobial peptide in a chloroplast genome and storing such peptide until release is necessary (Example 1, and page 10, lines 17-34).

## Response to §112, Second Paragraph Rejections

Applicant notes with appreciation the Examiner's helpful comments in regard to §112, second paragraph issues, and as a result of those helpful comments, Applicant has amended the claims to remove the §112, second paragraph rejections. Applicant responds to the §112, second paragraph rejections in the order to which they were given.

Claim 1 has been amended to provide proper antecedent basis and clear up any ambiguity in regard to the indefiniteness the Examiner has pointed out.

Claim 2 has been amended to place it in strict Markush format so that all members of the groups are now singular.

Claim 3 has been given a proper antecedent basis for the limitation of "the antimicrobial oligopeptide." Furthermore, Claim 3 has been amended to be placed in proper Markush format and is no longer indefinite as "PGLA" has been amended to "PGLa", which is supported by page 3, line 2 of Applicant's specification.

Claim 4 has been amended to depend from Claim 1.

Claim 5 has been amended to chnage the recitation of "wherein the selectable marker sequence is an antibiotic-free selectable marker" to read as "wherein the selectable marker sequence is not an antibiotic selectable marker sequence."

Claim 6 has been amended to clear up both antecedent basis issues and problems of indefiniteness.

Claim 7 has been amended in the recitation of "the progeny thereof" to clearly demonstrate that the progeny are related to the stably transformed plant. In Claims 7 and 13 the word "included" has been removed so as to render the claims definite.

In Claims 8-11 "which" has been replaced with "wherein the plant" to make the claims definite.

Claim 10 has been amended to insert a proper article after "is," and "plant" has been inserted after "cotton."

In Claims 12-13 "in which" has been replaced with "wherein."

Claim 12 has been given a proper antecedent basis for the limitation "all the chloroplasts" and "uniformly" has been replaced with "stably."

Claim 13 has been thoroughly amended to clear up antecedent basis issues and indefiniteness problems.

Claims 11and 14 have been canceled.

Applicant notes with appreciation the Examiner's helpful suggestions in regard to Claims 15 and 18 and, as a result, has amended Claim 15 in accordance with the Examiner's helpful suggestion. Furthermore, Claim 15 has been given proper antecedent basis and replaced "which" with "wherein the method".

Claims 16 and 17 are no longer in improper multiple dependent claim form. Claim 16 has been given an antecedent basis for the limitation "the target bacteria" and "the target micro" and "the bacteria" and, furthermore, the Applicant has amended Claim 16 to clear up its awkward and ungrammatical language.

Applicant respectfully submits that Claims 17 and 18 as amended do not need to show where the RBS and the 5' UTR are located relative to other components of the vector, because Applicant is merely claiming that the vector further comprises RBS and a 5' UTR, and one skilled in the art understands how to locate these areas on a plastid.

## Response to §103 Rejections

Applicant respectfully submits that solicited Claims 1-10, 12, 13 and 15-18 are patentable over Maliga et al. (1999, U.S. Patent 5,877,402) in view of Davies et al. (WO 90/11770). Applicant respectfully submits that Maliga et al. teaches away the insertion of a gene encoding for an antimicrobial peptide.

Maliga et al. teaches that foreign genes have been expressed in plastids to introduce novel traits, however, nothing disclosed by Maliga et al. indicates that the aforementioned foreign proteins could function *outside* of the plastids. In fact, no prior art suggests that an antimicrobial peptide could be produced inside the chloroplast, and

subsequently successfully exported and made to function outside of chloroplasts. It is noted that on page 6 of the Examiner's rejection, the Examiner has asserted "expressing pesticidal peptides in plants is unpredictable." Applicant notes that this assertion is consistent with the prior art belief that AMPs could not function properly within plants due to the inherent nature of antimicrobial/pesticidal peptides and their expression in plants.

In fact, in the past, no protein was shown to be produced inside the chloroplast and subsequently successfully exported so that it would be functional outside the chloroplast. Prior to Applicant's invention, there was no known method to study or test whether it was possible to express an antimicrobial peptide or any peptide in a chloroplast, and subsequently have that peptide function outside of the chloroplast. Applicant, however, has discovered that it is possible to export foreign proteins, specifically antimicrobial peptides produced in a plastid, to the cytosol. If one skilled in the art were to observe the teachings in Maliga et al., the skilled person would be inclined to believe that all foreign proteins must function within the transformed plastids.

A reference teaches away if it leaves the impression that the product would not have the property sought by the Applicant. *In re Calwell*, 138 USPQ 243,245 (CCPA 1963). If there is no motivation to make the invention, there is no suggestion of the combination. Contrary to prevailing wisdom, the Applicant has disclosed and shown through Example 1 of the specification, that it is possible to integrate a gene into a plastid that encodes for a peptide that functions outside of the plastid. It was previously believed in the prior art that AMPs were not stable inside living prokaryotic cells and, hence, unstable within plastids. As a result of the Applicant's discovery, however, it is possible to express several peptides within plastids, and subsequently transfer those peptides to the cytosol.

*In re Dembiczak*, 50 USPQ2d 614-617 (Fed. Cir. 1999) rearticulated the well-known principal that the Office cannot "use hindsight reconstruction to pick and choose

among isolated disclosures in the prior art to depreciate the claimed invention." It is the Patent Office that must show evidence of teaching or motivation to combine prior art references. *Id.* The Office must show evidence in the record that the prior art recognized that the successful integration of an AMP gene, transcribed in the plastid and subsequently functional outside of the plastid, was disclosed by the prior art. The Office must show that "a skilled artisan, confronted with the same problem as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for the combination in the manner claimed." *In re Rouffet*, 47 USPQ 2d 1456.

Prior to the Applicant's invention, no plastid genome had been stably transformed with a gene encoding for a peptide that exhibited antimicrobial activity. Applicant respectfully submits that Davies et al. does not teach a brassica plant transformed with a vector encoding either defensin or magainin. Davies et al. provide no indication that a gene encoding for an peptide could be, or should be inserted into a plant plastid genome. Example 5 of Davies et al. merely teaches the construction of a DNA sequence for the expression and transportation of the defensin MP-1. The MP-1 is then modified and introduced into the expression cassette which is then cloned into a binary vector (page 29, lines 7-10). The resulting construct is introduced into agrobacterium tumefaciens. This differs greatly from the claimed invention. Davies et al. does not teach an expression cassette used to transform a plastid genome. Further, the brassica plants of Davies et al. are transformed by the bacteria for only a single generation. The Applicant teaches a transformed plastid, which expresses the peptide in subsequent generations.

However, both Davies et al and Smith et al. (WO 99/06564) use eukaryotic elements for regulation of transgene expression. Such eukaryotic elements are non-functional in plastids. Davies et al and Smith et al. also use binary vectors for Agrobacterium mediated transformation (which take transgenes to the nucleus, away from the chloroplasts because of nuclear targeting sequences in binary vectors). There is

abundant evidence in the literature that binary vectors for Agrobacterium mediated transformation do not work for plastid transformation.

Claims 1-10, 12-13 and 15-18 have been rejected under 35 U.S.C. §103 based on Maliga et al. in view of Smith et al. (WO 99/06564). Applicant respectfully submits that the aforementioned claims as amended are patentable over Maliga et al. in view of Smith et al. Applicant respectfully submits that Smith et al. only merely states that an AMP can be expressed in plastids (Smith et al. page 5, lines 10-11). However, a study of the Smith et al. patent reveals that which was already well-known in the art, i.e. that the 35S promoter and the 35S enhancer of the Smith et al. patent are not functional in chloroplasts and as a result, Smith et al. does not show enablement for the expression of antimicrobial proteins in plastids. Further, as stated above, Smith et al not only use (non-functional) eukaryotic elements for regulation of transgene expression, but also use binary vectors for *Agrobacterium* mediated transformation.

Further, attention is directed to page 8, lines 11-15 of Smith where it is clear that the nucleic acid constructs encoding for the antimicrobial peptide gene are inserted into the nuclear genome of a plant cell. Applicant submits that it would not have been obvious to one of ordinary skill to transform a plant plastid with a vector encoding an antimicrobial peptide as described by Smith et al. As the Applicant has pointed out in the specification, modification of plant plastids is vastly different than the modification of the nuclear genome of the plant cell. Daniell (1999A) Nature Biotechnology 17: 855-856 teaches all known DNA delivery methods available to introduce foreign DNA into plastids.

> Plastid transformation was selected because of several advantages over nuclear transformation (Daniell, 1999 A, B; Bogorad, 2000; Heifetz, 2000). With concern growing about outcrossing of genetically altered genes, it should be noted that plastid expressed genes are maternally inherited in most crops. Gene containment is possible when foreign genes are engineered via the plastid genome, which prevents pollen transmission in crops that maternally inherit the plastid genome. Because a majority of

crop plants inherit their plastid genomes maternally, the foreign genes do not escape into the environment. (Page 3, lines 29-35 of this application.)

It is obvious to one of ordinary skill that there are a number of peptides made outside of a plant plastid but subsequently expressed in a plant plastid. It was not, however, obvious to engineer a plastid genome in such a way as to allow it to export foreign proteins into the cytosol. Until the Applicant's conceptual breakthrough, it was believed that microbes did not invade chloroplast compartments and, as a result, it would not have been beneficial to express antimicrobial peptides within a chloroplast compartment. The Applicant, however, discovered that microbes do reach the chloroplast compartment and that at the time of infection of the chloroplast they would lyse, releasing the antimicrobial peptide. Smith et al. teaches a gene coding for the cytosolic localized antimicrobial peptides but not peptides which are localized in the plastid and subsequently transported into the cytosol.

For the foregoing reasons set forth above, Applicant submits that the entire Application is now in condition for complete allowance, which action is respectfully requested.

Respectfully requested,

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